



STATE OF NEW HAMPSHIRE
DEPARTMENT OF HEALTH AND HUMAN SERVICES
DIVISION OF PUBLIC HEALTH SERVICES



NH Public Health Laboratories

How to Collect and Submit Clinical Specimens for Arboviral Testing

All suspect arbovirus cases should be reported to the Communicable Disease Control Section at 1-800-852-3345, ext. 4496 or the Public Health Laboratories at (603) 271-2764 or 271-3684 before specimens are submitted.

Diagnostic testing: The arboviral testing panel is a serological test for West Nile virus (WNV), Eastern Equine Encephalitis virus (EEE), St. Louis Encephalitis virus (SLE), and may include Powassan virus (depending on availability of reagents).

- The most efficient diagnostic method measures IgM antibodies in serum or CSF collected within 8 days of illness onset using the IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA).
- Since the IgM ELISA is a preliminary test, Plaque Reduction Neutralization test (PRNT) is required for case confirmation.
- Since IgM antibody does not cross the blood-brain barrier, IgM antibody in CSF strongly suggests central nervous system infection.
- Patients who have been recently vaccinated against or recently infected with related flaviviruses (e.g., yellow fever, Japanese encephalitis, dengue) may have positive WNV MAC-ELISA results. PRNT is required for case confirmation.

Specimens: Collect both CSF and serum, if possible.

- **Cerebrospinal fluid (CSF):** As early as the first few days of illness, IgM antibody can be demonstrated in CSF by antibody capture ELISA.

Since other viruses can cause encephalitis, culture for additional viruses (other arboviruses, enteroviruses, and herpesviruses) may be performed at the discretion of the laboratory.

Submit 2-5 ml in sterile, empty, screw-capped container. If less than 2 ml is received, testing will be prioritized as follows: IgM ELISA (PRNT confirmation if ELISA positive), and viral culture.

- **Serum:** Acute serum (3ml) should be collected and sent immediately to PHL for testing. Serum will be tested for IgM arboviral antibody. If specimen is IgM positive, then a convalescent specimen will be requested to determine the timing of infection.

Ideal timing of specimens for serology:

Specimen	Timing
Acute	3 to 10 days after onset of symptoms
Convalescent	2-3 weeks after acute sample

All spinal fluid submissions must be accompanied by a corresponding serum sample.

The following information is critical for accurate interpretation of test results and should be recorded on the accompanying case report form:

- Date of onset of disease symptoms
- Date of specimen collection
- Unusual immunological status of patient (e.g. immunosuppression)
- Brief clinical summary including suspected diagnosis (e.g., encephalitis or meningoencephalitis)
- Current address and travel history to flavivirus-endemic areas
- History of prior vaccination against flavivirus disease (e.g., yellow fever, Japanese encephalitis, or Central European encephalitis)
- Disease history (e.g., previous history of viral encephalitis or Dengue fever)

Procedure for submission of serum or CSF:

1. Perform lumbar puncture or venipuncture (SST or whole blood tube) by standard aseptic technique.
2. Label the specimen tubes with patient's full name and the date of collection.
3. If possible, centrifuge blood to separate serum.
4. For CSF, tightly seal cap and then wrap parafilm around seal to provide additional protection from leakage during transport.
5. Fill out requisition form completely, being sure to request "arboviral serology" or "viral culture (CSF)".
6. Place CSF inside zip-lock biohazard bag and seal.
7. Place blood tube inside inner metal liner. Be sure there is enough absorbent material to cushion tubes in transit or to absorb liquid in case of leaking or broken tubes. Cap liner tightly.
8. Wrap the requisition form around the OUTSIDE of the inner metal liner.
9. Insert the metal liner into the outer cardboard container, and cap tightly. Make certain that the mailing container is labeled with the name and address of the NH PHL.
10. Send with courier or call PHL for special pickup after hours. Refrigerate at 2-8 °C if is not possible to send specimen immediately.

The arboviral collection kit consists of:

- ❖ A labeled cardboard outer mailing container
- ❖ An aluminum inner liner
- ❖ An SST vacutainer blood collection tube
- ❖ A polypropylene tube and parafilm for transport of CSF
- ❖ Absorbent material
- ❖ Requisition form

To order specimen collection kits, please call 271-4661, or 1-800-852-3345, extension 4661.

For further technical information regarding diagnostic testing, please call Sue MacRae, Virology/STD, at 271-2764, or 1-800-852-3345, extension 2764.